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***Marine Pollution Bulletin* 2017, 122(1-2), 297-305.**

Marine Pollution Bulletin 122 (2017) 297–305

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

# Anthropogenic noise compromises the anti-predator behaviour of the European seabass, *Dicentrarchus labrax* (L.)

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ARTICLE INFO

*Keywords:*
Marine noise
Predator avoidance
Pile driving
Drilling
Marine construction
Broadband noise

ABSTRACT

Anthropogenic noise is a significant pollutant of the world's oceans, affecting behavioural and physiological traits in a range of species, including anti-predator behaviours. Using the open field test, we investigated the effects of recordings of piling and drilling noise on the anti-predator behaviour of captive juvenile European seabass in response to a visual stimulus (a predatory mimic). The impulsive nature of piling noise triggered a reflexive startle response, which contrasted the behaviour elicited by the continuous drilling noise. When presented with the predatory mimic, fish exposed to both piling and drilling noise explored the experimental arena more extensively than control fish exposed to ambient noise. Fish under drilling and piling conditions also exhibited reduced predator inspection behaviour. Piling and drilling noise induced stress as measured by ventilation rate. This study provides further evidence that the behaviour and physiology of European seabass is significantly affected by exposure to elevated noise levels.

## 1. Introduction

The levels and distribution of anthropogenic sound in the oceans have increased over the past sixty years in line with growth of the maritime industries (Ellison et al., 2012). Piling and drilling are among the activities that contribute to low frequency underwater noise, particularly in coastal areas. Broadband noise generated from piling is impulsive and high intensity (Bailey et al., 2010) whereas drilling creates continuous sounds (Broudic et al., 2014). Underwater noise in the low frequency range overlaps the hearing sensitivity of many fish species (Popper and Fay, 2011). Detrimental impacts are predicted for species that utilise sound for ontogenetic behaviours such as mate finding and courtship, as well as routine behaviours including species recognition, foraging, and predator-prey interactions (Codarin et al., 2009; Picciulin et al., 2010; Purser and Radford, 2011; Bracciali et al., 2012; Voellmy et al., 2014a; Shannon et al., 2016; Simpson et al., 2016). However, knowledge gaps remain concerning the ultimate endpoint of noise-induced behavioural modification at both individual and population levels.

Startle and avoidance reactions are key prey survival responses in a predator-prey situation (Webb, 1986). Noise can impact prey risk assessment as a result of reallocation of the prey's finite attention (Dukas, 2004), distracting it and preventing it from responding to predation threat (Chan et al., 2010; Simpson et al., 2015). Increased noise levels can impair the threat perception of the prey fish, potentially compromising escape (reviewed in Slabbekoorn et al., 2010). Conversely, prey may increase anti-predator vigilance and exploratory behaviour; actions which may have energy budget implications (Shannon et al., 2016). Noise can act as a stressor and may lead to altered activity and locomotion patterns (Mendl, 1999).

The European seabass, *Dicentrarchus labrax* has increasingly been used in the study of anthropogenic noise effects on fish. The hearing sensitivity of seabass is most acute at low frequencies (100–1000 Hz; Lovell, 2003); coincident with many anthropogenic noises in water (Götz et al., 2009). The scale of the behavioural responses depends on the nature of the noise (Neo et al., 2014). Increases in motility and changes in swimming performance in juveniles have been reported in response to synthetic continuous (Buscaino et al., 2010) and impulsive sounds (Neo et al., 2015). Regarding anti-predator behaviour, Everley et al. (2015) have shown that exposure to playback piling noise can reduce responsiveness to a visual stimulus. Further, startle responses are known to occur after exposure to low frequency sounds (Kastelein et al., 2008). Changes in physiological and biochemical parameters were also found in response to exposure to low frequency impulsive and continuous noise (Santulli et al., 1999; Buscaino et al., 2010; Bruintjes, 2013; Bruintjes et al., 2014; Debusschere et al., 2016).

In the current study we investigated the effects of recorded piling and drilling noise on seabass physiology and anti-predator behaviour. We hypothesised that this additional noise would result in an increased number of startle responses and increased motility, compared to

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http://dx.doi.org/10.1016/j.marpolbul.2017.06.067
Received 14 April 2017; Received in revised form 21 June 2017; Accepted 21 June 2017
Available online 26 June 2017

ambient conditions, with altered responses to predator attack.

## 2. Materials and methods

### 2.1. Ethics statement

All procedures were approved by the Newcastle University animal-related research/work, operating outside the Animals (Scientific Procedures) Act 1986 (for vertebrates only) and followed the University of Exeter Ethics Committee (2013/247: Impacts of global change on aquatic organisms) approved protocols.

### 2.2. Study species and holding conditions

Fifty-four seabass (47.94 ± 9.09 g; 14.52 ± 1.70 cm) sourced from a commercial hatchery (Ecloserie Marine, Gravelines, France) were housed in a fiberglass holding tank (150 L × 80 W × 50 H cm) with a water depth of 30 cm (water volume = 360 L) in the Aquatic Resource Centre at the University of Exeter. In the holding tank water was refreshed continuously with a closed recirculating system and oxygen was maintained by a cylindrical ceramic airstone placed close to the water pump. Fish were fed pellets (Skretting Perla MP-L, Italy) twice daily and maintained within a 12-h photoperiod and water temperature of 16.5 °C. Individual fish were transferred in a net from the holding tank to the experimental tank, where each fish was given a thirty minute acclimation period.

### 2.3. Noise playback preparation and assessment

Drilling noise recordings were made in August 2014 between 17:00 and 17:30 at Yarmouth (Cowes, Isle of Wight, N50.70950, W1.51666) during the installation of one of four screw piles supporting a tidal device. Three different recordings were used to create the playback drilling noise tracks. Recordings included vessel noise (a 720 HP/530 Kw @ 2000 rpm twin engine multicat-type vessel, length overall = 20 m, beam overall = 7 m) and seabed drilling noise. Seabed sediments were characterised by course, medium and fine gravel with clay nearer the surface. Underwater sounds were recorded using a C55 Cetacean Research Technology hydrophone (Transducer Sensitivity + Preamplifier Gain – Effective Sensitivity: -165 dB, re: 1 V/μPa) connected to a Fostex FR-2LE compact audio recorder (20 Hz − 20 KHz ± 2 dB; FS 44.1/48 kHz, calibrated against a 1 KHz reference tone of known amplitude). Recordings were made five metres below the sea surface.

Three recordings of piling carried out in Swansea Bay during the installation of a lifeboat station (Swansea Bay, N51.56989, W3.97401) were used to create the playback piling noise tracks. A 1.2 m diameter monopile was hammered at 20–30 m into the sea bed. The recordings were made 2–5 m below the sea surface using a calibrated hydrophone (HTI 99HF; sensitivity without preamp: − 183 dB re: 1 V/μPa) connected to an EASDA 14 data logger (Rtsys, France). Three ambient noise recordings without vessel noise or sudden sounds were made at the same site when piling was not in operation. These were used to create the playback ambient noise tracks.

Noise samples of ten seconds duration were band-pass filtered from 0.1 to 3 KHz (FFT 1024, Hann window) using Avisoft SAS Lab Pro vs 5.2.08 (Avisoft Bioacoustics, Berlin, Germany) in order to play within the underwater speaker specifications and to minimize resonant frequencies within the experimental tank (Akamatsu et al., 2002). Each filtered noise file was looped forming a repeating 30 min playback track using Audacity (http://audacity.sourceforge.net). Drilling tracks included a ten seconds fade in and out, to simulate the gradual increase in noise level recorded in the field. There was no fade in or fade out to the piling track as piling noise has a sudden onset. Ambient noise playback tracks included five seconds fade in and out. Fade in and out times were shorter as the maximum amplitudes were lower than for drilling and piling noise tracks (Fig. 1).

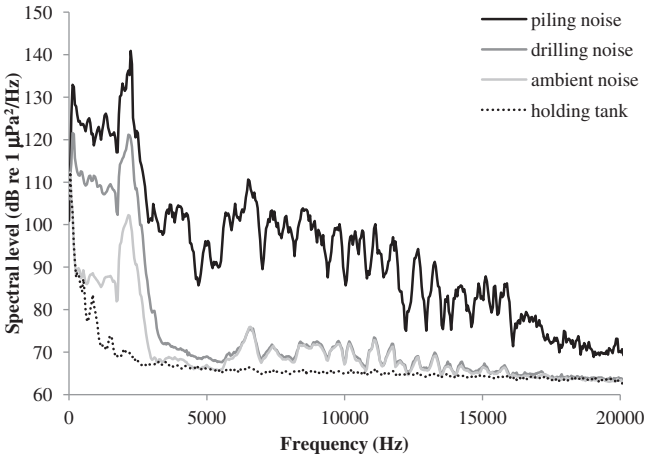


**Fig. 1.** Acoustic conditions in the experimental tank. Mean sound pressure spectral levels (units normalized to 1 Hz, Hann window, FFT length 1024, 50% overlap) during band-pass filtered piling (1 s averages), drilling and ambient noise (30 s averages) playbacks. An example of the holding tank recording is given for comparison.

Noise tracks were played back through a portable audio recorder (Sony PCM-M10; frequency ranges 20 Hz–20 KHz), amplifier (Kemo Electronic GmbH; 18 W; frequency response range: ~40–20,000 Hz), and an Aqua30 underwater loudspeaker (DNH; effective frequency range 80–20,000 Hz). Sound pressure in the experimental tank was recorded during playback using a calibrated hydrophone (HTI 96-MIN; manufacturer-calibrated sensitivity − 164.3 dB re 1 V/μPa; frequency range 2–30,000 Hz) suspended mid-water in the centre of the tank (10 cm from the bottom). Before starting the experiment, playback recordings were adjusted in Audacity to achieve uniform sound pressure levels within noise treatments. The mean peak sound pressure of the piling tracks (averaged from one second recordings during pile strikes) was 152 ± 3.5 dB RMS re 1 μPa; the drilling tracks had a mean sound pressure of 132 ± 0.42 dB RMS re 1 μPa (averaged from 30 s recordings) and the mean sound pressure of the ambient tracks (averaged from 30 s recordings) was 117 ± 1.00 dB RMS re 1 μPa. A comparison between noises recorded in the field and playback noise is shown in the electronic supplementary material (Fig. S1 and S2; Table S1).

Like most fishes, hearing in *D. labrax* may be dominated by the particle motion element of sound (Popper and Fay, 2011), but because they have a swim bladder they are also likely to be sensitive to changes in pressure (Wysocki et al., 2009). For logistical reasons we only report the sound pressure levels of the playback of recordings for comparison between noise treatments. Particle motion levels in the experimental arena were also measured in the middle of the water column. Electronic supplementary material shows example particle acceleration levels (Fig. S3).

### 2.4. Experiment set-up and protocol

One tank (54.8 L × 45.1 W × 45.2 H cm; water depth 20 cm) made from 6 mm thick glass with a 10 mm base placed on a fiberglass bench was used for all experimental trials (Fig. S4). Thick polystyrene blocks placed between the tank and bench were used to reduce vibrations. The underwater speaker was placed at the bottom of the tank facing upward, centred and suspended beneath a 3 mm thick white Perspex© false bottom. The speaker was wrapped in medium density laminated polyethylene foam to avoid additional vibrations. A video camera (Panasonic HC-V700) was mounted above the tank and recorded the whole experiment. Perspex© panels with anti-glare sides were used to reduce reflections. A fourth wall had a window left uncovered to allow the fish to see the looming predator.

A spherical blue squash ball (40 mm diameter) fixed to a clear

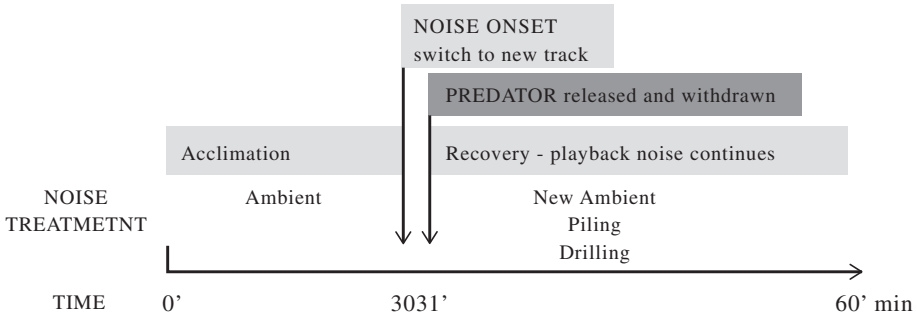


**Fig. 2.** Flow diagram of the experimental set-up.

pendulum arm moving through 45° to a position next to the glass but without contacting the tank was used as a looming visual stimulus (a simulated predator) (Everley et al., 2015; Simpson et al., 2015; Fig. S4). The 'predator' was only visible to the fish once it was released.

In the experimental tank each fish was given a 30 min acclimation period during which ambient noise (CONTROL) was played (Fig. 2). After acclimatisation, playback was switched to either a drilling (DRILLING) or piling (PILING) noise track or to a different ambient (AMBIENT) noise track. After one minute the looming predator was released and withdrawn; during this time the fish were kept under the same noise conditions as experienced during predator looming. Each playback run lasted 60 min. Fish behaviour was recorded throughout the experiment. Individuals were tested once only in an independent-samples design. Nine combinations of 'CONTROL to CONTROL' or 'CONTROL to DRILLING' or 'CONTROL to PILING' noise were created and randomly played to the 54 fish.

### 2.5. Behavioural observations and analysis

The open field test (OFT) was used to explore fish movement, exploratory behaviour and other fear- and anxiety-related behaviours (Champagne et al., 2010). Video tracking and computation used EthoVision XT 9.0 (Noldus, The Netherlands; © 2015 Noldus Information Technology). Video footage was analysed continuously and variables were computed over one minute.

Different variables of interest were chosen to analyse the fish behaviour:

1. Velocity (V; cm/s): this was used as a measure of general activity and was computed as the mean distance moved by the centre point of the fish per unit time (60 s) between two consecutive X-Y coordinates. Swimming speed was predicted to increase in response to pure tones (600 Hz tone pulse; Kastelein et al., 2007; 0.1–1 KHz linear sweep; Buscaino et al., 2010).
2. Distance moved (DM; cm): this quantified swimming activity and was computed as the mean distance moved per unit time (60 s). A minimum distance threshold between two consecutive points of 0.003 cm was set. Distance moved was expected to decrease after a stimulus (Millot et al., 2009).
3. Meandering (M; absolute; deg/cm): this provided an indicator of the speed of directional change and quantified the swimming path complexity. A set minimum distance criterion of 0.003 cm was applied. A high value of this parameter is expected in fish engaged in local searching behaviours (Maximino et al., 2010).
4. Turn angle (TA; absolute; deg): this measured the rate of change of the angle made by the anterior portion of the fish (snout to centre of mass) and was computed as the difference between two subsequent values for heading direction. In seabass the turn angle was expected to increase in response to a predator (Benhaïm et al., 2013).
5. Thigmotaxis (Z1, Z2; s): this quantified the spatial distribution of the fish over time. It was measured as the time spent by each individual in defined areas of the experimental arena (Z1: centre, Z2 edge; Fig. S5). The time from the start of noise playback until the fish moved to Z2 and the total number of occurrences in Z2 was computed. Movements between areas were expected to change in response to a stimulus (Domenici, 2001), and thigmotaxis (tendency to spend more time in Z2 than Z1) was expected to increase as a flight response toward a threatening stimulus (Millot et al., 2009).

### 2.6. Restoring behaviour during settling

Behaviour was observed to establish whether the seabass had settled during the acclimation period. Fish behaviour was examined considering two sequences of one minute: the first minute of the initial acclimation period (SETTLING) versus the last minute (29–30 min).

### 2.7. Playback noise effects – 1 min control/1 min noise conditions

Whether or not the fish startled (c-start type response) to the onset of noise playback was determined 'blind' by the investigator (IS) i.e. with muted sound. Two sequences of one minute were considered for each recording: one sequence before noise onset (CONTROL) and one sequence after noise onset (NOISE) for each treatment.

### 2.8. Anti-predator response – 1 min noise/1 min predator conditions

Behavioural data were analysed blind and included: 1) whether the fish showed a startle response or not in response to the predator, and 2) the response latency. Two sequences of one minute were considered, one sequence before predator appearance (NOISE) and one sequence after (PREDATOR) for each treatment.

The arena was divided into four areas of equal size (25% of the total arena; Fig. S6). The PREDATOR ZONE was defined as the area closest to the tank wall from which the predator loomed at the fish; the other areas were at fixed distances from the predator and the SAFE ZONE was the zone furthest from the predator. Three parameters were measured to assess whether fish exposed to different noise treatments showed a difference in inspection behaviour: 1) the time spent by individual fish in the two areas, 2) the latency of the first inspection of the predator area, and 3) how often the fish visited the predator zone (frequency of occurrence).

### 2.9. Recovery in noise conditions – 27 min playback

To test immediate versus delayed effects of the playback noise on anti-predator response, each behavioural variable was measured every minute from the minute after predator appearance until the end of the experiment (from minutes 32–59); their distributions were examined over the entire period (PLAYBACK). In addition, the distributions of these variables at the end of the experiment (minute 59–60) were compared with the one minute time sequence before the playback trial (CONTROL) to assess whether the fish had restored their behaviour to a level comparable to the acclimatisation period.

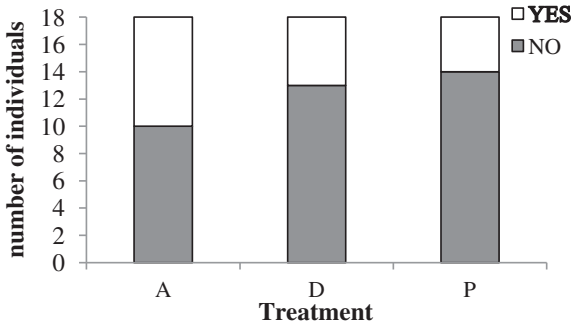


**Fig. 3.** Number of fish that showed a startle response to a simulated predatory attack during playback of different noise treatments. A = ambient, D = drilling, P = piling noise (n = 18 per treatment).

### 2.10. Physiological effects

Opercula beat rate (OBR) was used as a proxy for the physiological state of the fish in response to noise playback and the looming predator. Opercula beats were scored independently and blind by the investigator (IS) from 15 s video recordings. If the beats could not be observed counting was paused and resumed within the one minute sequence (Simpson et al., 2015). Beat counts were multiplied by four to obtain per minute OBR.

### 2.11. Statistical analysis

All data were analysed for normality (Shapiro – Wilk test) and homoscedacity of variance (Levene's test). A series of Wilcoxon signed ranks tests were used on the behavioural measures computed at two time sequences: 1 min SETTLING vs 1 min 29–30 min to test whether fish had settled in the arena before playback experiments.

To test for behavioural changes during the trials, five bins of exposure sequence from the data set were considered: 1 min before sound exposure (CONTROL), the first minute of exposure (NOISE), the first minute after the predator loomed (PREDATOR), the 27 min after the predator loomed (PLAYBACK) and the last minute of noise exposure (END). Changes in the behavioural variables computed with EthoVision between two time sequences, 1 min CONTROL vs 1 min NOISE were analysed by Wilcoxon signed ranks tests. A Kruskal Wallis test determined differences in behavioural measures between playback noise treatments (1 min NOISE). Wilcoxon signed ranks tests were used for the thigmotaxis analysis as the tank areas were not independent.

A Chi-squared test was used to assess differences in frequency of reflexive startle response to the looming predator between noise treatments; a Kruskal Wallis test was used to assess the difference in time to startle. A Wilcoxon signed ranks test was used to analyse the immediate behavioural changes between 1 min PREDATOR and 1 min NOISE sequences, with Kruskal Wallis and Mann-Whitney *U* tests to compare behavioural variables between treatments.

Wilcoxon Signed Ranks Tests and Kruskal Wallis tests were used to assess differences in the time spent in SAFE and PREDATOR ZONES between treatments. Latency to predator detection and frequencies of inspection of the predator zone were analysed by Kruskal Wallis tests.

To assess the recovery responses all behavioural measures were examined over the entire 27 min main playback period (PLAYBACK) using Kruskal Wallis tests, followed by Mann-Whitney *U* tests for pairwise comparisons. To assess whether fish restored behaviour, the behavioural measures computed at the end of the playback trials (END) were compared with CONTROL using Wilcoxon signed ranks tests. A Kruskal Wallis test, followed by Mann-Whitney *U* tests, was used to investigate differences between noise treatments.

OBR data were normally distributed and had equal variances; therefore, a repeated measures ANOVA with Huynh-Feldt correction was used within time sequences (CONTROL, NOISE, PREDATOR, END) with OBR as the dependent variable and time sequence within-subject (repeated) factors, followed by Bonferroni *post-hoc* tests. Independent sample *t*-tests were used to compare OBR between treatments.

All tests were computed using IBM SPSS 22 software (IBM Corp. Released 2013. Armonk, NY: IBM Corp.). All reported p-values are two-tailed and results were considered significant at an alpha value of 0.05.

## 3. Results

### 3.1. Settling to the novel environment

All fish restored their normal behaviour during the acclimation period (1 min SETTLING vs 1 min 29–30 min; all p-values $< 0.001$; Table S2); however, thigmotaxis was different with the fish spending more time at the tank edges compared to the centre (all p-values $> 0.05$).

### 3.2. Playback noise effects

When the sound track was switched from ambient noise to another noise treatment no fish subjected to ambient or drilling noise showed a startle response compared with seven of the 18 fish exposed to piling noise. There was no significant impact of noise treatment on the behavioural measures compared to ambient conditions. Thigmotaxis continued to be significant with fish spending more time in Z2 than Z1 ($n_{ambient} = n_{drilling} = n_{piling} = 18$; all p-values $< 0.05$). There were no significant differences in exploration behaviour and movement between treatments.

### 3.3. Anti-predator response

There was no statistical difference in the startle response to the visual stimulus (predator mimic) between treatments ($\chi^2 = 2.232$, df = 2, n = 54, p = 0.328; Fig. 3). The time delay before the startle response occurred was similar among noise treatments ($\chi^2 = 2.052$, df = 2, $n_{ambient} = 8$, $n_{drilling} = 5$, $n_{piling} = 4$, p = 0.633).

Fish exposed to playback ambient noise had a significantly reduced velocity (V; 1 min NOISE vs 1 min PREDATOR: $n_{noise} = n_{predator} = 18$, Z = 2.15, p = 0.031; Fig. 4); whereas fish exposed to playback drilling and piling noise did not. Fish exposed to ambient noise playback had a significantly decreased distance travelled (1 min NOISE vs 1 min PREDATOR: $n_{noise} = n_{predator} = 18$, Z = 2.24, p = 0.025; Fig. 4), whereas fish exposed to drilling and piling noise did not. No significant difference in fish V and DM was found between treatments ($p > 0.05$).

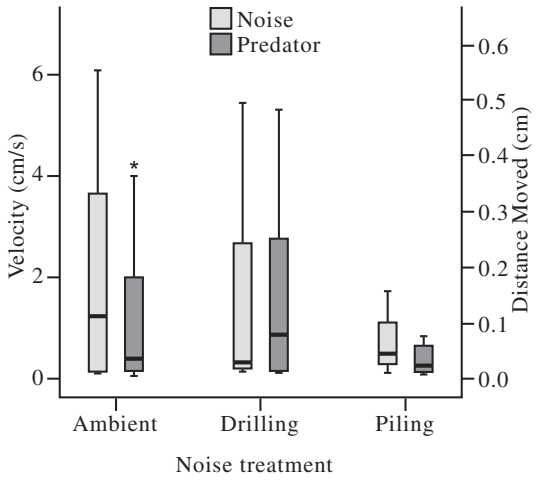


**Fig. 4.** Velocity and distance moved (median ± IQ) of *Dicentrarchus labrax* exposed to ambient noise during the time sequences before and after the visual stimulus. * represents significant difference ($p < 0.05$).

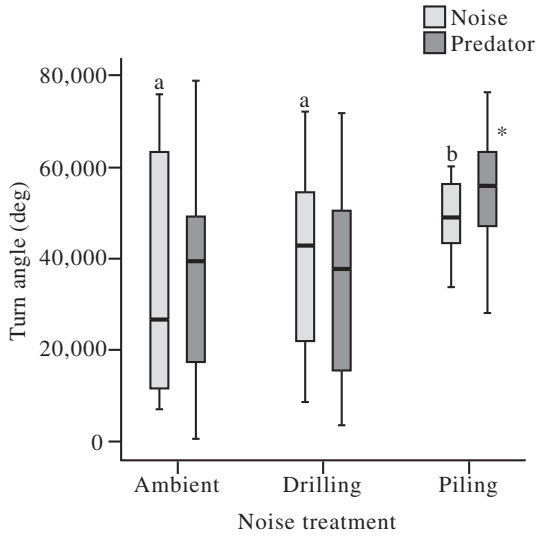


**Fig. 5.** Turn angle (median ± IQ) of *Dicentrarchus labrax* during the time sequences before and after the visual stimulus. * represents significant difference between time sequences ($p < 0.05$). Different subscripts represent significant difference between noise treatments ($p < 0.05$).

When presented with the visual predatory stimulus a significant difference in fish turn angle (TA) was found between treatments ($\chi^2 = 6.28$, df = 2, n = 54, $p = 0.043$). Fish exposed to piling noise had a significantly increased TA (1 min NOISE vs 1 min PREDATOR: $n_{noise} = n_{predator} = 18$, $Z = 2.83$, $p = 0.039$; Fig. 5), showing significantly more turns than fish exposed to ambient ($U = 99$, $p = 0.046$) and drilling noise ($U = 89$, $p = 0.021$).

Thigmotaxis reduced in the first minute after the appearance of the predator compared to the first minute of noise, but this reduction was not apparent in the piling noise treatment ($\chi^2 = 9.18$, df = 2, $p = 0.010$) where fish spent more time close to the tank edge compared with both ambient noise ($U = 111$, $p = 0.034$) and drilling ($U = 78$, $p = 0.002$).

Fish in ambient and drilling noise conditions spent significantly more time in the SAFE ZONE compared to the PREDATOR ZONE (ambient: $Z = 2.200$, $p = 0.028$; drilling: $Z = 2.386$, $p = 0.017$), whereas fish under piling conditions did not ($Z = 0.883$, $p = 0.377$). There was no difference in the time spent in the two zones between noise treatments ($\chi^2 = 9.077$, df = 2, adj $p = 0.336$). There were also no differences in the latency period to predator detection ($\chi^2 = 0.221$, df = 2, adj $p = 0.895$) or the frequency of movements to the PREDATOR ZONE ($\chi^2 = 0.866$, df = 2, adj $p = 0.649$) between treatments.

### 3.4. Recovery of anti-predator behaviour in noisy conditions

Over the entire playback period the difference in TA between treatments remained significant ($\chi^2 = 123.06$, df = 2, adj $p < 0.001$). Fish exposed to drilling and piling noise had a higher TA than fish in ambient conditions (ambient vs drilling: $U = 97{,}015$, $p < 0.001$; ambient vs piling: $U = 83{,}429.5$, $p < 0.001$) while piling noise exposed fish had a higher TA than fish exposed to drilling noise ($U = 112{,}203.5$, $p < 0.001$). At the end of the playbacks the fish exposed to piling noise did not have a reduced TA compared to the control (1 min CONTROL vs 1 min END: $Z = 0.196$, $p = 0.845$), whereas fish exposed to drilling noise did ($Z = 2.249$, $p = 0.025$).

Significant differences were found in DM between noise treatments over the main playback ($\chi^2 = 28.037$, df = 2, adj $p < 0.001$) with fish exposed to ambient noise travelling significantly further than fish in piling and drilling conditions (ambient vs piling, $U = 117{,}105$, $p < 0.001$; ambient vs drilling, $U = 109{,}913$, $p < 0.001$). Fish exposed to piling noise travelled significantly further than fish under drilling conditions ($U = 118{,}434$, $p = 0.004$). At the end of the experiments the fish had similar DM values to the control conditions (1 min END vs 1 min CONTROL; ambient: $Z = 2.60$, $p = 0.795$; drilling: $Z = 1.207$, $p = 0.227$; piling: $Z = 0.588$, $p = 0.557$). By the last minute of playback there was no longer any significant treatment effect ($\chi^2 = 2.201$, df = 2, adj $p = 0.333$).

Thigmotaxis was still significant for fish exposed to any of the playback treatments (ambient: $Z = 2.253$, $p = 0.024$; drilling: $Z = 2.839$, $p = 0.005$; piling: $Z = 2.652$, $p = 0.008$). Fish exposed to piling noise spent more time close to the tank edge compared with both ambient ($U = 104{,}824$, $p < 0.001$) and drilling noise ($U = 111{,}875.5$, $p < 0.001$) treatments. For fish exposed to drilling noise, this difference was significant with fish spending more time close to the edge than fish in ambient noise conditions ($U = 122{,}346$, $p = 0.035$). By the end of the experiments the fish subjected to drilling and piling noise had similar thigmotaxis responses to those in control conditions (1 min END vs 1 min CONTROL; drilling: $Z = 1.255$, $p = 0.209$; piling: $Z = 1.789$, $p = 0.074$), whereas fish in ambient noise significantly reduced the time spent in Z2 (1 min END vs 1 min CONTROL: $Z = 2.198$, $p = 0.028$). There was no noise treatment effect at the end of the trials ($\chi^2 = 1.626$, df = 2, adj $p = 0.444$).

Over the main playback the fish remained for longer in the zone farthest from the predator (all p-values < 0.001), with no significant difference between noise treatments ($\chi^2 = 2.226$, df = 2, adj $p = 0.329$). The frequency of detection of the predator zone was significantly different between the treatments ($\chi^2 = 32.128$, df = 2, adj $p < 0.001$), with fish exposed to drilling and piling noise approaching the predator zone less frequently than fish in ambient noise conditions (ambient vs drilling, $U = 113{,}718$, $p < 0.001$; ambient vs piling, $U = 111{,}934.5$, $p < 0.001$). The latency period to predator detection was significant between treatments ($\chi^2 = 24.408$, df = 2, adj $p < 0.001$), with fish in drilling noise requiring less time to detect the predator than fish in both ambient ($U = 114{,}375.5$, $p < 0.001$) and piling conditions ($U = 119{,}709.5$, $p = 0.002$). There was no noise treatment effect on the time spent in the two zones at the end of the playbacks ($\chi^2 = 1.011$, df = 2, adj $p = 0.603$).

Over the entire playback period the fish exposed to drilling and piling noise started to reduce erratic swimming behaviour (meander, M). The difference between treatments was significant ($\chi^2 = 51.433$, df = 2, adj $p < 0.001$) with fish exposed to piling and drilling noise showing less erratic swimming than fish in ambient noise conditions (ambient vs piling: $U = 107{,}070$, $p < 0.001$; ambient vs drilling: $U = 101{,}779.5$, $p > 0.001$). By the end of the playbacks many of the fish exposed to drilling and piling noise had started to show meander comparable to that of ambient fish ($\chi^2 = 3.936$, df = 2, adj $p = 0.140$). Meander values did not return below control conditions (all p-values > 0.05).

### 3.5. Opercula beat rate (OBR)

Drilling and piling noise affected the physiology of the fish (rANOVA; drilling: $F = 11.028$, df = 3, $p < 0.001$; piling: $F = 7.231$, df = 3, $p = 0.001$; Fig. 6). Pairwise comparisons (adjusted with a Bonferroni correction and equal variances assumed) revealed that during playback of drilling noise the fish had significantly increased mean OBR both at the onset of playbacks (1 min CONTROL vs 1 min NOISE; $p = 0.001$) and after the predator attack (1 min NOISE vs 1 min PREDATOR; $p < 0.001$). Fish exposed to piling noise had significantly increased OBR at the onset of noise playback (1 min CONTROL vs 1 min NOISE; $p = 0.005$), but not when the predator was released (1 min NOISE vs 1 min PREDATOR; $p = 0.096$). By the end of the trials fish exposed to drilling had a significantly reduced OBR (1 min PREDATOR vs 1 min END; $p = 0.037$) similar to levels of control fish (1 min END vs 1 min CONTROL; $p = 0.989$). Fish exposed to piling noise maintained sustained OBR until the end of the trials (Fig. 6).

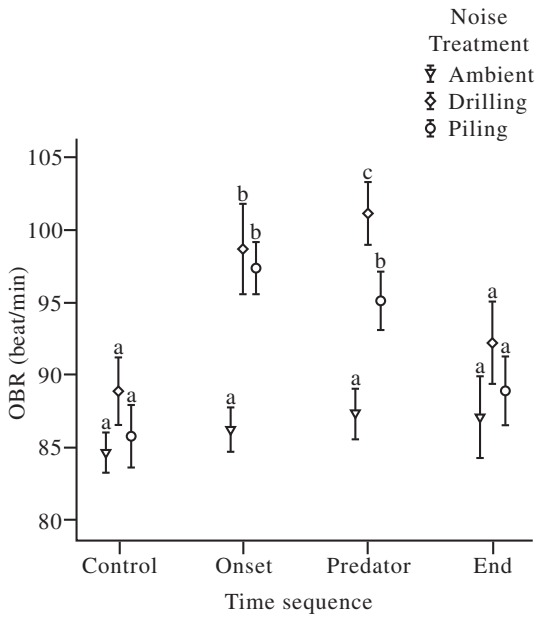


**Fig. 6.** OBR values (mean ± SE) of *Dicentrarchus labrax* scored at each time sequence during exposure to noise treatments. Different superscripts indicate significant differences ($p < 0.05$).

Fish exposed to playback of drilling noise had a significantly higher mean OBR than fish in ambient noise, both at the onset of playback (independent samples *t*-test; n = 18 for each treatment; t(34) = 3.6; p = 0.01; equal variances not assumed) and after predator release (independent samples *t*-test; n = 18 for each treatment; t(34) = 4.9; p < 0.001; equal variances assumed). Likewise, fish exposed to piling noise had a significantly higher mean OBR than fish in ambient noise at playback onset (independent samples *t*-test; n = 18 for each treatment; t(34) = 4.7; p < 0.001; equal variances assumed) and after predator release (independent samples *t*-test; n = 36; t(34) = 2.9; p = 0.006; equal variances assumed). No significant differences were found in the mean OBR between drilling and piling noise at each time point (independent samples *t*-tests; n = 18 for each treatment; all p-values > 0.05).

## 4. Discussion

### 4.1. Seabass response to playback noise treatments

The onset of playback piling noise induced a startle response in juvenile *Dicentrarchus labrax*. Conversely, exposure to drilling or ambient noise did not elicit any immediate response. The differing responses to the noise treatments may be explained by acoustic properties of the respective stimuli (e.g. rise time and intensity level). Piling noise was characterised by a rapid rise time to the highest pressure value that would have impacted the fish without warning (e.g. in 0.04 s a single strike reached 164 dB re 1 μPa zero-to-peak), thus triggering a startle response, whereas the ambient and drilling noise conditions were characterised by a gradual increase in level that was faded in. Little is known about organismal responses to impulsive underwater sounds that differ in rise times, but this component has been suggested to affect fish physiology and behaviour (reviewed in Hawkins et al., 2015); the results of the present study would appear to lend support to that hypothesis.

The startle response is an involuntary action mediated by a pair of hindbrain Mauthner neurones. The acoustic properties of piling noise will have stimulated the Mauthner cells, thereby triggering the reflexive escape behaviour. In contrast, the acoustic properties of the ambient and drilling conditions bore greater similarity to the background acoustic conditions of the holding tank, and were evidently insufficiently high in level to initiate the startle reflex (Szabo et al., 2006). This suggests that in this species the involuntary responsiveness to sound could be affected only when the average sound pressure level significantly elevated relative to the background. This conclusion is supported by Neo et al. (2014) wherein a consistent amplitude noise (SPL of 165 dB re 1 μPa) was sufficiently high in level to trigger a startle response. Kastelein et al. (2007) made similar observations wherein *D. labrax* reacted more strongly to acoustic pingers operating at higher source levels.

The increase in ventilation rate, determined as the opercula beat rate (OBR), indicated that fish exposed to playback of anthropogenic noise experienced higher stress levels than fish in the ambient noise control. This agrees with Radford et al. (2016) in relation to piling noise, however seabass exposed to continuous noise (shipping sounds) did not exhibit elevated OBR. Further support comes from Santulli et al. (1999), where stress-related biomarkers were observed to change in caged seabass after air-gun firing. Exposure to impulsive sound evidently elicits physiological stress in seabass due to its intermittent nature (Wysocki et al., 2006; Neo et al., 2014; Nichols et al., 2015), yet no distinction was made between OBR response to impulsive and continuous sound in the present study. Therefore, it may be suggested that although piling noise triggers reflex behaviours, the responses would appear not to be detrimental to the fish.

None of the behavioural measures related to exploration, swimming activity or anxiety were affected by playback noise onset. Exploration behaviour is an important feature in fish as it leads to finding food, mates and escapes routes, whereas anxiety-related behaviours are innate responses to a potential threat (Blaser et al., 2010; Maximino et al., 2010). Captive fish respond to acute stress differently from wild fish by lessening the behavioural responses (Malavasi et al., 2004). This implies that under natural conditions this species would potentially alter its behaviour differently than what was found in this experiment. Wild fish are free to move away from the noise source which may dampen its impact.

### 4.2. Anti-predator response in noisy conditions

Exposure to drilling and piling noise affected the anti-predator response of *D. labrax* compared to ambient noise conditions. Control fish became motionless in response to the looming predator, significantly decreasing their mean swimming velocity and distance moved. Fish subjected to piling noise exhibited increased turning behaviour when challenged, but without moving to the area furthest from the predator (safe zone). In contrast, fish subjected to drilling noise increased the time spent in the safe zone.

Immobility is a widespread anti-predator behaviour across different taxa (O'Brien and Dunlap, 1975; Lima and Dill, 1990; Giaquinto and Volpato, 2001). In this study, immobility appeared to be the preferred response for seabass when the predator became visible, as fish exposed to ambient noise adopted this strategy. That fish exposed to anthropogenic noise did not favour immobility indicates a modification of the anti-predator behaviour brought about by noise exposure.

The differences in turn angle between treatments reflected a higher state of vigilance (alertness) in fish exposed to piling noise. This measure is a typical indicator of fish risk assessment (Millot et al., 2009) and suggests that exploration increased when exposed to anthropogenic noise. This likely resulted from exposure to a higher sound level (Bégout Anras and Lagardere, 2004). Increased alertness due to noise-induced stress was corroborated by the increase in OBR in fish exposed to playback of anthropogenic noise. However, whereas fish exposed to drilling noise had a significantly increased OBR when the predator loomed, fish exposed to piling did not. This may be a result of fish exposed to piling having already had a significantly increased OBR at the onset of playback, thus carrying the stress-induced effects forward to when the predator appeared.

It was predicted that seabass exposed to additional noise would show increased latency times to respond to a predatory stimulus compared to the ambient noise treatment (Chan et al., 2010; Voellmy et al., 2014b; Simpson et al., 2015), with fish exposed to piling noise showing a greater effect than the other noise treatments (Stahlman et al., 2011). This was not the case because the time delay before the startle response occurred was similar among noise treatments; similar findings were reported by Everley et al. (2015). The present study shows that the additional noise (piling and drilling playbacks) affects the kinematic component of the anti-predator response (swimming path and velocity, including turning) more than the behavioural component (responsiveness and response latency). Further information is needed to determine whether this is a consistent characteristic of the seabass anti-predator response under noise or context-specific exposure, with a need to balance the costs and benefits of a rapid escape response (Domenici, 2010).

The fish recovered a higher level of swimming activity thirty minutes after visual stimulation, exhibiting an increased distance travelled and decreased swimming complexity, suggesting that the fish began to recover homogeneous swimming activity in the tank. However, the levels of certain variables (e.g. turning) did not return to those measured prior to predator looming, which indicates that fish remained alert to predation (Millot et al., 2009). Over the course of playback, fish exposed to piling and drilling maintained higher levels of vigilance compared to ambient noise conditions, but by approaching the predator area less frequently they also reduced their predator inspection behaviour. This may be due to distraction by the noise. These findings are in accord with Radford et al. (2016) who showed that over time (12 weeks), seabass adapted to exposure to impulsive noise either through a process of familiarisation or a shift in hearing threshold. The differences observed between the current study and Radford et al. (2016) are most likely related to the respective exposure periods (short-term, current study vs long-term exposure, Radford et al., 2016). This serves to highlight the importance of longer duration exposures when studying impulsive sound impacts.

Late, and shorter-lasting effects in fish exposed to drilling noise were identified as the fish increased their turning rate and reduced their spatial distribution (spending a longer time in the safe zone) compared to the ambient treatment over the main playback period; the turning measure significantly decreased at the end of the experiment. On the other hand, fish exposed to piling noise showed acute and longer-lasting effects with increased turning within the minute after the predator loomed, and had consistently higher behavioural measures than ambient until the end of the trial. Increased turning could also be maladaptive and energetically costly for this species as it could increase the drag during continuous swimming and impair their manoeuvrability during other activities, such as predation or migration (Bone, 1975; Blake et al., 1995). Sustained exploration could increase predation risk (Dugatkin, 1992), although the effects could be mitigated by being part of a group, through combined vigilance (Lima and Bednekoff, 1999).

The increased OBR measured within the first minute could indicate a more general allostatic stress response (McEwen and Wingfield, 2003). However, elevated stress levels could also lead to a reduction in reactivity and thus to the initiation of the anti-predator response (Mendl, 1999). This could explain why seabass in drilling noise conditions showed higher turning than ambient with a certain delay.

All fishes are able to use their otoliths to detect the particle motion component of sound (Radford et al., 2012). Sound pressure can be detected by fish from pressure-induced oscillations of compressible structures, such as the swim bladder (Popper and Fay, 2011). In seabass the swim bladder appears soon after hatching (Chatain, 1986), therefore, it is reasonable to assume that this species be able to detect both particle motion and pressure changes of the sound (Radford et al., 2012). If seabass are close to the seabed they may well be affected by seabed vibration (Hazelwood, 2012; Hazelwood and Macey, 2015, 2016).

There are both acoustic and behavioural limitations to tank studies (reviewed in Carroll et al., 2016) which can be summarise as follows: 1) reflections from tank walls may transform acoustic fields increasing near-field effects (invalidation of the particle motion relationship to pressure); 2) the acoustic fields in tanks may differ from those that occur in the animals' natural environment, and 3) the artificial experimental conditions to which animals are exposed may make interpretation and extrapolation difficult. According to Gray et al. (2016), using bigger tanks does not guarantee better acoustic conditions, especially if low frequency sounds are employed. Also bigger space, i.e. basins, may be subject to unpredictable boundary interactions that transform the acoustic field (Hubert et al., 2016). For these reasons, the findings from our study should be seen as conservative: particle motion levels relative to sound pressure in the experimental arena were higher than that expected in open fields and may be representative of levels only close to the acoustic source. In addition, because we used an underwater speaker, the streamed signals had altered characteristics compared to the original recordings, although analysis of spectral content showed that characteristics of the original recordings were partially retained in playbacks and they differed between noise types. Finally, the loudspeaker could not replicate lower frequencies (to which seabass might respond) and ground vibrations associated with the impact of piling on the seabed (and potentially from the drilling vessel), which could affect seabass when it is close to the sea bottom. Therefore, this study shows that near to the sound source there are impacts of acoustic disturbance with implications for survival, which may affect fish that remain in the proximity of the anthropogenic acoustic source (near-field). This could interest those fish that have high fidelity to the site where the activities are carried out (Iafrate et al., 2016). How distant from the sources these effects could go depends on the propagation loss induced by environment. Further research in the open ocean where the propagation of acoustic energy varies with the physical properties of the environment (e.g. substrates, water depths) will increase our knowledge of the effects found in this study.

This study directly demonstrates the potential for acoustic disturbances to have similar effects, even when they differ in their regime (impulsive vs continuous) and levels. However, the ecological realism of studies carried out in experimental tanks needs to be carefully considered when extending interpretations to the field. In addition to the different acoustic conditions to which the fish are exposed, the behavioural responses to noise may also be altered by the experimental conditions. Fish in the wild, for example, could leave the affected area; something that our fish could not do. In addition, over a longer exposure, fish could lessen their responses or even cease to respond to the same type of noise (Radford et al., 2016). Integrating both field and laboratory studies using the same playback methods may be employed to confirm that responses of seabass found in this study is not simply an artefact of acoustic conditions that occur in the tank environment (Simpson et al., 2015). In addition, integrating playback experiments in tanks and in open water conditions employing real anthropogenic sources could establish impact thresholds in the context of realistic exposure levels (Simpson et al., 2016).

The present experiment, carried out under laboratory conditions with captive fish, allowed for control over confounding factors due to domestication (Malavasi et al., 2004; Malavasi et al., 2008) or lack of learning experience (Kelley and Magurran, 2003) as all fish were hatchery born and reared under the same conditions. Moreover, the experiment used fish with similar weight and fed under the same regime to limit any influence on risk-taking behaviour due to higher growth rate or food needs (reviewed in Domenici, 2010). Studies on wild versus domesticated fish have shown that anti-predator behaviour is sensitive to artificial rearing (Malavasi et al., 2008; Benhaïm et al., 2012), but that some innate anti-predator responses (e.g. inspection behaviour) remained in hatchery-reared fish (Malavasi et al., 2004). It can therefore be concluded that the observed responses in this study were initiated by exposure to playback noise.

### 4.3. Management implications

This study provides further direct evidence that the behaviour and physiology of *D. labrax* is affected by exposure to elevated levels of noise, and that the effects differ significantly from ambient noise. Even a moderate increase in sound level had an effect on physiology. The results show that elevated noise has the potential to induce higher vigilance toward a visual stimulus, possibly as a consequence of increased anxiety; the higher intensity level of piling noise has an immediate effect on seabass behaviour. This study also demonstrates that recovery following predator encounter is more likely to happen for fish exposed to drilling noise than piling noise, but the fitness consequences of exposure to anthropogenic noise on the prey are similar between the two sound sources. Exposure to drilling and piling noise reduced predator inspection and potentially compromised threat recognition.

There are proposals to mitigate the effects of anthropogenic noise by reducing the level of such noise in the sea (Boyd et al., 2011) and, indeed, the present results indicate that impact could be mitigated by using drilling instead of piling (Broudic et al., 2014) to reduce immediate effects such as startling. In this case, drilling of one screw pile lasted < 30 min and the effects on the surrounding fish were probably limited due to the short time employed and the lower sound levels produced compared to piling. However, activities that use piling or drilling in the sea require excavation of deep wells and installation of arrays that can last for days with short periods of inactivity. It therefore cannot be excluded that a longer noise exposure could bring detrimental effects on survival, albeit that Radford et al. (2016) showed no long-term harmful effects. A viable option, evidenced by this study, could be to drill for short periods of time, allowing resting between sessions, therefore minimising any deleterious effects on local fish populations, bearing in mind that the acoustic conditions encountered in the field could change and sound exposure thresholds of a given response must be assessed, for both sound pressure and particle motion components of the sound source.

## Acknowledgements

We thank Merin Broudic for the original piling recordings and the Natural Environment Research Council (NE/L014335/1) for funding the original drilling recordings. We thank the University of Exeter for hosting this study and the Aquatic Resource Centre staff (Jan, Steve, and Greg) for technical support. We are grateful to Irene Voellmy and Rick Bruintjes of the University of Bristol for assistance and useful discussions; Sophie Nedelec and Steve Simpson for lending the acoustic equipment; and the School of Marine Science and Technology for additional funding support.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.06.067.

## References

Akamatsu, T., Okumura, T., Novarini, N., Yan, H.Y., 2002. Empirical refinements applicable to the recording of fish sounds in small tanks. J. Acoust. Soc. Am. 112, 3073–3082.

Bailey, H., Senior, B., Simmons, D., Rusin, J., Picken, G., Thompson, P.M., 2010. Assessing underwater noise levels during pile-driving at an offshore windfarm and its potential effects on marine mammals. Mar. Pollut. Bull. 60, 888–897.

Bégout Anras, M.-L., Lagardere, J.-P., 2004. Domestication et comportement chez les poissons téléostéens. Prod. Anim. 17, 211–215.

Benhaïm, D., Péan, S., Lucas, G., Blanc, N., Chatain, B., Bégout, M.-L., 2012. Early life behavioural differences in wild caught and domesticated sea bass (*Dicentrarchus labrax*). Appl. Anim. Behav. Sci. 141, 79–90.

Benhaïm, D., Bégout, M.L., Chatain, B., 2013. Unfamiliar congener used as a visual attractor in wild caught 1and domesticated sea bass (*Dicentrarchus labrax*) placed in a T-maze. J. Aquacult. Res. Dev. 4, 169–175.

Blake, R., Chatters, L., Domenici, P., 1995. Turning radius of yellowfin tuna (*Thunnus albacares*) in unsteady swimming manoeuvres. J. Fish Biol. 46, 536–538.

Blaser, R.E., Chadwick, L., McGinnis, G.C., 2010. Behavioral measures of anxiety in zebrafish (*Danio rerio*). Behav. Brain Res. 208, 56–62.

Bone, Q., 1975. Muscular and energetic aspects of fish swimming. In: Wu, T.Y.T., Brokaw, C.J., Brennen, C. (Eds.), Swimming and Flying in Nature, vol. 2. Springer US, Boston, MA, pp. 493–528.

Boyd, I.L., Frisk, G., Urban, E., Tyack, P., Ausubel, J., Seeyave, S., Cato, D., Southal, B., Weise, M., Andrew, R., Akamatsu, T., Ling, R.D., Erbe, C., Farmer, D., Gentry, R., Gross, T., Hawkins, A., Li, F., Metcalf, K., Miller, J.H., Moretti, D., Rodrigo, C., Shinke, T., 2011. An international quiet ocean experiment. Oceanography 24, 174–181.

Bracciali, C., Campobello, D., Giacoma, C., Sara, G., 2012. Effects of nautical traffic and noise on foraging patterns of Mediterranean damselfish (*Chromis chromis*). PLoS One 7 (7), e40582.

Broudic, M., Voellmy, I., Dobbins, P., Robinson, S., Berggren, P., Laing, S., Blake, L., Radford, A., Lepper, P., Pace, F., 2014. Underwater noise emission from the NOAH's drilling operation at the NAREC site, Blyth, UK. In: Proceedings of the 2nd International Conference on Environmental Interactions of Marine Renewable Energy Technologies (EIMR2014). Blyth, UK, April 2014.

Bruintjes, R., 2013. Predicting movement of fishes in response to anthropogenic noise. In: NERC MREKE Internship report.

Bruintjes, R., Renshaw, E., Rossington, K., Armstrong-Smith, E., Tozer, B., Jones, D., Botterell, Z., Benson, T., Simpson, S.D., 2014. A tool to predict the impact of anthropogenic noise on fish. In: 2nd International Conference on Environmental Interactions of Marine Renewable Energy Technologies (EIMR2014). 28 April – 02 May 2014, Stornoway, Isle of Lewis, Outer Hebrides, Scotland.

Buscaino, G., Filiciotto, F., Buffa, G., Bellante, A., Stefano, V.D., Assenza, A., Fazio, F., Caola, G., Mazzola, S., 2010. Impact of an acoustic stimulus on the motility and blood parameters of European sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.). Mar. Environ. Res. 69, 136–142.

Carroll, A., Przeslawski, R., Duncan, A., Gunning, M., Bruce, B., 2016. A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pollut. Bull. 114, 9–24.

Champagne, D.L., Hoefnagels, C.C.M., de Kloet, R.E., Richardson, M.K., 2010. Translating rodent behavioral repertoire to zebrafish (*Danio rerio*): relevance for stress research. Behav. Brain Res. 214, 332–342.

Chan, A.A.Y.-H., Giraldo-Perez, P., Smith, S., Blumstein, D.T., 2010. Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biol. Lett. 6, 458–461.

Chatain, B., 1986. La vessie natatoire chez *Dicentrarchus labrax* et *Sparus auratus*. Aquaculture 53, 303–311.

Codarin, A., Wysocki, L.E., Ladich, F., Picciulin, M., 2009. Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). Mar. Pollut. Bull. 58, 1880–1887.

Debusschere, E., Hostens, K., Adriaens, D., Ampe, B., Botteldooren, D., De Boeck, G., De Muynck, A., Sinha, A.K., Vandendriessche, S., Van Hoorebeke, L., 2016. Acoustic stress responses in juvenile sea bass *Dicentrarchus labrax* induced by offshore pile driving. Environ. Pollut. 208, 747–757.

Domenici, P., 2001. The scaling of locomotor performance in predator–prey encounters: from fish to killer whales. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 131, 169–182.

Domenici, P., 2010. Context-dependent variability in the components of fish escape response: integrating locomotor performance and behavior. J. Exp. Zool. A Ecol. Genet. Physiol. 313, 59–79.

Dugatkin, L.A., 1992. Tendency to inspect predators predicts mortality risk in the guppy (*Poecilia reticulata*). Behav. Ecol. 3, 124–127.

Dukas, R., 2004. Causes and consequences of limited attention. Brain Behav. Evol. 63, 197–210.

Ellison, W., Southall, B., Clark, C., Frankel, A., 2012. A new context-based approach to assess marine mammal behavioral responses to anthropogenic sounds. Conserv. Biol. 26, 21–28.

Everley, K.A., Radford, A.N., Simpson, S.D., 2015. Pile-Driving Noise Impairs Anti-Predator Behaviour of the European sea Bass *Dicentrarchus labrax*. In: Popper, A.N., Hawkins, A.D. (Eds.), The Effects of Noise on Acquatic Life II. Springer, New York, pp. 273–279.

Giaquinto, P.C., Volpato, G.L., 2001. Hunger suppresses the onset and the freezing component of the antipredator response to conspecific skin extract in pintado catfish. Behaviour 138, 1205–1214.

Götz, T., Hastie, G., Hatch, L.T., Raustein, O., Southall, B.L., Tasker, M., Thomsen, F., 2009. Overview of the impacts of antropogenic underwater sound in the marine environment. In: OSPAR Biodiversity Series, London, pp. 134.

Gray, M.D., Rogers, P.H., Popper, A.N., Hawkins, A.D., Fay, R.R., 2016. "Large" tank acoustics: how big is big enough? In: Popper, A.N., Hawkins, A. (Eds.), The Effects of Noise on Aquatic Life II. Springer New York, New York, NY, pp. 363–369.

Hawkins, A.D., Pembroke, A.E., Popper, A.N., 2015. Information gaps in understanding the effects of noise on fishes and invertebrates. Rev. Fish Biol. Fish. 25, 39–64.

Hazelwood, R.A., 2012. Ground roll waves as a potential influence on fish: measurement and analysis techniques. In: Popper, A., Hawkins, A. (Eds.), The Effects of Noise on Aquatic Life II. Springer Science + Business Media, pp. 449–452.

Hazelwood, R.A., Macey, P., 2015. The intrinsic directional information of ground roll waves. In: Popper, A., Hawkins, A. (Eds.), The Effects of Noise on Aquatic Life, II. Springer Science + Business Media, pp. 447–453.

Hazelwood, R.A., Macey, P., 2016. Modeling water motion near seismic waves propagating across a graded seabed, as generated by man-made impacts. J. Mar. Sci. Eng. 4, 471–4714.

Hubert, J., Neo, Y.Y., Campbell, J.A., Slabbekoorn, H., 2016. Particle motion and pressure

soundscape in outdoor vs indoor set-up. In: Proceedings of Meetings on Acoustics 4ENAL 27, 070007. Dublin, Ireland 10-16 July 2016.
Iafrate, J.D., Watwood, S.L., Reyier, E.A., Scheidt, D.M., Dossot, G.A., Crocker, S.E., 2016. Effects of pile driving on the residency and movement of tagged reef fish. PLoS One 11, e0163638.
Kastelein, R.A., van der Heul, S., van der Veen, J., Verboom, W.C., Jennings, N., de Haan, D., Reijnders, P.J.H., 2007. Effects of acoustic alarms, designed to reduce small cetacean bycatch in gillnet fisheries, on the behaviour of North Sea fish species in a large tank. Mar. Environ. Res. 64, 160–180.
Kastelein, R.A., van der Heul, S., Verboom, W.C., Jennings, N., van der Veen, J., de Haan, D., 2008. Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. Mar. Environ. Res. 65, 369–377.
Kelley, J.L., Magurran, A.E., 2003. Learned predator recognition and antipredator responses in fishes. Fish Fish. 4, 216–226.
Lima, S.L., Bednekoff, P.A., 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. Am. Nat. 153, 649–659.
Lima, S.L., Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68, 619–640.
Lovell, J.M., 2003. The hearing abilities of the bass, *Dicentrarchus labrax*. In: Technical report commissioned by ARIA Marine Ltd. for the European Commission Fifth Framework Programme. Project Reference: Q5AW-CT-2001-01896.
Malavasi, S., Georgalas, V., Lugli, M., Torricelli, P., Mainardi, D., 2004. Differences in the pattern of antipredator behaviour between hatchery-reared and wild European sea bass juveniles. J. Fish Biol. 65, 143–155.
Malavasi, S., Georgalas, V., Mainardi, D., Torricelli, P., 2008. Antipredator responses to overhead fright stimuli in hatchery-reared and wild European sea bass (*Dicentrarchus labrax* L.) juveniles. Aquac. Res. 39, 276–282.
Maximino, C., de Brito, T.M., da Silva Batista, A.W., Herculano, A.M., Morato, S., Gouveia, A., 2010. Measuring anxiety in zebrafish: a critical review. Behav. Brain Res. 214, 157–171.
McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15.
Mendl, M., 1999. Performing under pressure: stress and cognitive function. Appl. Anim. Behav. Sci. 65, 221–244.
Millot, S., Bégout, M.-L., Chatain, B., 2009. Exploration behaviour and flight response toward a stimulus in three sea bass strains (*Dicentrarchus labrax* L.). Appl. Anim. Behav. Sci. 119, 108–114.
Neo, Y.Y., Seitz, J., Kastelein, R.A., Winter, H.V., ten Cate, C., Slabbekoorn, H., 2014. Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biol. Conserv. 178, 65–73.
Neo, Y.Y., Ufkes, E., Kastelein, R.A., Winter, H.V., ten Cate, C., Slabbekoorn, H., 2015. Impulsive sounds change European seabass swimming patterns: influence of pulse repetition interval. Mar. Pollut. Bull. 97, 111–117.
Nichols, T.A., Anderson, T.W., Širović, A., 2015. Intermittent noise induces physiological stress in a coastal marine fish. PLoS One 10, e0139157.
O'Brien, T.J., Dunlap, W.P., 1975. Tonic immobility in the blue crab (*Callinectes sapidus*, Rathbun): its relation to threat of predation. J. Comp. Physiol. Psychol. 89, 86–94.
Picciulin, M., Sebastianutto, L., Codarin, A., Farina, A., Ferrero, E.A., 2010. In situ behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789; fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758; fam. Pomacentridae) living in a marine protected area. J. Exp. Mar. Biol. Ecol. 386, 125–132.
Popper, A.N., Fay, R.R., 2011. Rethinking sound detection by fishes. Hear. Res. 273, 25–36.
Purser, J., Radford, A.N., 2011. Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). PLoS One 6 (2), e17478.
Radford, C.A., Montgomery, J.C., Caiger, P., Higgs, D.M., 2012. Pressure and particle motion detection thresholds in fish: a re-examination of salient auditory cues in teleosts. J. Exp. Biol. 215, 3429–3435.
Radford, A.N., Lèbre, L., Lecaillon, G., Nedelec, S.L., Simpson, S.D., 2016. Repeated exposure reduces the response to impulsive noise in European seabass. Glob. Chang. Biol. 22, 3349–3360.
Santulli, A., Modica, A., Messina, C., Ceffa, L., Curatolo, A., Rivas, G., Fabi, G., D'Amelio, V., 1999. Biochemical responses of European sea bass (*Dicentrarchus labrax* L.) to the stress induced by off shore experimental seismic prospecting. Mar. Pollut. Bull. 38, 1105–1114.
Shannon, G., McKenna, M.F., Angeloni, L.M., Crooks, K.R., Fristrup, K.M., Brown, E., Warner, K.A., Nelson, M.D., White, C., Briggs, J., McFarland, S., Wittemyer, G., 2016. A synthesis of two decades of research documenting the effects of noise on wildlife. Biol. Rev. 91, 982–1005.
Simpson, S.D., Purser, J., Radford, A.N., 2015. Anthropogenic noise compromises antipredator behaviour in European eels. Glob. Chang. Biol. 21, 586–593.
Simpson, S.D., Radford, A.N., Nedelec, S.L., Ferrari, M.C.O., Chivers, D.P., McCormick, M.I., Meekan, M.G., 2016. Anthropogenic noise increases fish mortality by predation. Nat. Commun. 7, 10544.
Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., Popper, A.N., 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427.
Stahlman, W.D., Chan, A.A.Y.-H., Blumstein, D.T., Fast, C.D., Blaisdell, A.P., 2011. Auditory stimulation dishabituates anti-predator escape behavior in hermit crabs (*Coenobita clypeatus*). Behav. Process. 88, 7–11.
Szabo, T.M., Weiss, S.A., Faber, D.S., Preuss, T., 2006. Representation of auditory signals in the M-cell: role of electrical synapses. J. Neurophysiol. 95, 2617–2629.
Voellmy, I.K., Purser, J., Flynn, D., Kennedy, P., Simpson, S.D., Radford, A.N., 2014a. Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Anim. Behav. 89, 191–198.
Voellmy, I.K., Purser, J., Simpson, S.D., Radford, A.N., 2014b. Increased noise levels have different impacts on the anti-predator behaviour of two sympatric fish species. PLoS One 9 (7), e102946.
Webb, P., 1986. Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Micropterus salmoides*). Can. J. Fish. Aquat. Sci. 43, 763–771.
Wysocki, L.E., Dittami, J.P., Ladich, F., 2006. Ship noise and cortisol secretion in European freshwater fishes. Biol. Conserv. 128, 501–508.
Wysocki, L.E., Codarin, A., Ladich, F., Picciulin, M., 2009. Sound pressure and particle acceleration audiograms in three marine fish species from the Adriatic Sea. J. Acoust. Soc. Am. 126, 2100–2107.